Final Report

Optimal surgical therapy in a porcine *(Sus scrofa)* model of extra-thoracic penetrating trauma resulting in hemorrhagic shock: ED thoracotomy vs. immediate trans-abdominal vascular control

A porcine model for evaluating the management of non-compressible torso hemorrhage

8 November 2010

Reporting Period: 12 December 2008 - 8 November 2010

Prepared for
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DISTRIBUTION STATEMENT A
Approved for public release: distribution unlimited.

UNCLASSIFIED

20101115031

REPORT DOCUMENTATION PAGE

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
11-08-2010	Final Report	Dec 2008 - Nov 2010

4. TITLE AND SUBTITLE
Optimal surgical therapy in a porcine (Sus scrofa) model of extra-thoracic penetrating trauma resulting in hemorrhagic shock: ED thoracotomy vs. immediate trans-abdominal vascular control

A porcine model for evaluating the management of non-compressible torso hemorrhage

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
DISTRIBUTION STATEMENT A: Approved for public release: distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This report introduces a standardized large animal model of non-compressible torso hemorrhage which permits assessment of different management strategies for this relatively common and extremely challenging combat injury pattern. Class IV hemorrhagic shock was induced in anesthetized adult swine through an iliac arterial injury and animals were randomized to different treatment groups. Following vascular control, the injury was shunted, and damage control resuscitation was performed. Serum markers, intravenous fluid volumes, and vasopressor requirements were tracked. Post-mortem tissue analysis was performed to compare levels of acute ischemic injury between groups. After model development (n=6), treatment animals underwent non-compressible hemorrhage with thoracic aortic clamping (n=6), supra-celiac aortic clamping (n=6), direct vascular control (n=6), and endovascular aortic occlusion (n=6). This study presents a large animal model of class IV hemorrhagic shock from non-compressible hemorrhage which permits comparison of various vascular control methods to address this challenging problem. Future studies utilizing this standardized model will allow further development of strategies for the management of non-compressible hemorrhage.

15. SUBJECT TERMS
Non-compressible hemorrhage, emergency thoracotomy, combat injury, large animal model, vascular injury

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	UU		Jeremy W. Cannon, MD, SM, FACS
U	U	U			19b. TELEPHONE NUMBER (Include area code) 210-736-2558

A porcine model for evaluating the management of non-compressible torso hemorrhage

Short title: Porcine model of non-compressible hemorrhage

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Background: Non-compressible hemorrhage from central vascular injuries remains the leading cause of preventable death in modern combat. This report introduces a large animal model of non-compressible torso hemorrhage which permits assessment of the various approaches to this problem.

Methods: Yorkshire swine (mean weight = 80.9 kg) were anesthetized and monitoring devices for central aortic pressure, carotid flow, and intracerebral and transcutaneous brain oximetry were applied. Class IV hemorrhagic shock was induced through an iliac arterial injury and animals were randomized to different vascular control methods including thoracic aortic clamping, supra-celiac aortic clamping, direct vascular control, and proximal endovascular balloon occlusion. Following vascular control, the injury was shunted, and damage control resuscitation was performed. Serum markers, intravenous fluid volumes, and vasopressor requirements were tracked over a subsequent resuscitation period. Post-mortem tissue analysis was performed to compare levels of acute ischemic injury between groups.

Results: The protocol for animal preparation, hemorrhage volume, open surgical technique and post-hemorrhage resuscitation protocol was developed using four animals. The endovascular approach was developed using two additional animals. After model development, treatment animals subsequently underwent non-compressible hemorrhage with thoracic aortic clamping (n=6), supra-celiac aortic clamping (n=6), direct vascular control (n=6), and endovascular aortic occlusion (n=6). Premature death occurred in one animal in the direct vascular control group.

Conclusion: This study presents a large animal model of class IV hemorrhagic shock from non-compressible hemorrhage which permits comparison of various vascular control methods to address this challenging problem. Future studies utilizing this standardized model will allow

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further development of strategies for the management of non-compressible hemorrhage.

Introduction

The optimal approach to moribund patients following penetrating abdominal trauma is hotly debated in both civilian and military practice.[1, 2] The most recent practice management guidelines remain vague on the optimal treatment approach in this situation due to limited clinical evidence.[3] In 1976, Ledgerwood and colleagues first described a tamponade effect from the acute abdominal compartment syndrome in several patients with penetrating torso trauma that was released with laparotomy.[4] Thus, they advocated for a preemptive thoracotomy in the operating room for proximal aortic control in such cases. This treatment algorithm is now initiated during the initial assessment in the Emergency Department in patients who present in profound shock following penetrating torso trauma. However, with the ability to initiate damage control resuscitation measures and rapidly transport such a patient to a nearby operating room for definitive intervention, this practice bears re-examination. Furthermore, advances in endovascular techniques which enable minimally invasive and rapid proximal aortic control permit the exploration of a potential new treatment paradigm in this situation. As an initial step to future investigations in this area, a clinically relevant large animal model of non-compressible hemorrhage with hemorrhagic shock resulting gin physiologic exhaustion is required. This manuscript describes such a model developed to further analyze the question of optimal control methods for non-compressible hemorrhage from penetrating extra-thoracic injuries.

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Materials and Methods

Study design

Yorkshire swine (*Sus scrofa*) with a mean weight of 80.9 kg were subjected to large-volume controlled hemorrhage from an iliac artery injury and were randomized to various treatment strategies (Fig. 1). These treatments included both open vascular control measures as well as an endovascular approach; so model development was required for the overall preparation as well as for each treatment strategy. The different strategies included early resuscitative thoracotomy with thoracic aortic clamping, trans-abdominal supra-celiac aortic clamping, direct vascular control of the injury site without aortic clamping and endovascular balloon occlusion of the thoracic aorta. Outcomes measured to evaluate the differences between groups included cerebral perfusion (carotid flow and partial pressure of brain tissue oxygenation), central perfusion (central arterial pressure), laboratory analysis (serum pH, base deficit, lactate), resuscitation requirements (intravenous fluid volume and vasopressor requirement), and histologic evaluation. This study was approved by the Institutional Animal Care and Use Committee, and all procedures were conducted in animal research facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International under the guidance and supervision of the institution's licensed veterinarians and support staff.

Animal preparation

Anesthesia was induced utilizing ketamine and maintained with 2-4% inhaled isoflurane. All study animals underwent identical preparatory procedures to include exposure of the right external iliac artery, exposure of the right common carotid artery and jugular vein, and exposure of the right brachial artery (Fig. 2). Additionally, all study animals underwent direct brain tissue oxygenation monitoring through right frontal cranial burr hole. A supra-umbilical peritoneal

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infusion catheter was inserted to permit simulation of hemoperitoneum. Animals randomized to thoracic aortic clamping also underwent thoracotomy with thoracic aortic exposure while those randomized to supra-celiac aortic clamping underwent exposure of the supra-celiac aorta. Animals in the endovascular balloon occlusion group underwent placement of an additional left femoral arterial line.

Partial pressure of brain tissue oxygenation monitoring and transcutaneous infrared brain oximetry monitoring

A cerebral oximetry probe (LICOX®, Integra LifeSciences, Plainsboro, NJ) was placed in all animals to quantify the change in cerebral oxygenation throughout this study. To facilitate placement, animals were first positioned prone. The optimal insertion point is midway between the superior brow and inferior aspect of the ear in the anterior-posterior direction and the midway between the saggital midline and lateral-most aspect of the skull in the lateral direction (Fig. 2, inset). A hand drilled burr hole was created at this point and the probe inserted and calibrated. Additionally, a transcutaneous infrared brain oximetry monitor (Pediatric SomaSensor®, Somanetics Corporation, Troy, MI) was positioned across the frontal scalp of the animal. The probe was calibrated and periodic values were obtained at standardized time-points. The animal was then placed in the supine position for the remainder of the protocol.

Vascular exposure and monitoring lines

A right neck dissection was performed for placement of a carotid flow probe (Transonic Flowprobe®, Transonic Systems Inc., Ithaca, NY) and jugular central venous catheter. Once the carotid sheath was opened and both vessels exposed, the carotid flow probe was positioned around the common carotid artery. The flow probe was calibrated and periodic values were

obtained at standardized time-points. A central venous catheter was placed into the jugular vein using a modified Seldinger technique.

A 5 Fr microcatheter was placed into the brachial artery for hemodynamic monitoring and to facilitate required laboratory draws. This catheter was inserted along the medial aspect of the front limb. Using the palpable pulse as a landmark, the brachial artery was dissected out sharply through a longitudinal incision. Care was taken to avoid the small lateral branches, and silk ties were used to isolate the vessel. The distal vessel was ligated and this tie was used for traction to insert the micro needle and guidewire in a modified Seldinger fashion. The catheter was then inserted and secured in place using the distal silk tie. Additional anchoring sutures were then placed to avoid catheter dislodgement.

Retroperitoneal exposure of right external iliac vessels

The iliac vessels were exposed using a standard retroperitoneal approach through a low midline laparotomy incision. The peritoneal sac can be elevated off the abdominal sidewall using gentle blunt dissection. Once the iliac artery and vein were exposed, the cephalad lymphatic tissue overlying the artery was swept medially. Proximal and distal exposure was obtained and vessel loops were placed around the external iliac artery. An 8.5 Fr introducer sheath was placed in the iliac artery using a modified Seldinger technique. This catheter insertion site served as the source of simulated non-compressible torso hemorrhage in all groups.

Placement of an intraperitoneal infusion catheter

To simulate intra-abdominal hemorrhage, two liters of saline were delivered into the animal's peritoneal cavity. To avoid the midline urinary bladder, the peritoneal cavity was accessed

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through a small supraumbilical midline incision. Intravenous infusion tubing was passed into the peritoneal cavity and secured at the skin.

Pre-hospital hemorrhage

A standardized method for blood volume estimation and the appropriate rate of hemorrhage was utilized to create a significant pathophysiologic response.[5, 6] For the hemorrhage rate and volume calculations, the animal's weight was multiplied by an average estimate of total blood volume in adult swine of 66 mL/kg which gave the approximate total blood volume in mL.[7] The target hemorrhage volume was weight based at 35% of the total blood volume to yield a hemorrhagic shock state from an exsanguinating injury. Pre-hospital hemorrhage time was set at 20 minutes across all groups with half of the calculated target hemorrhage volume removed over the first seven minutes and the remainder of the hemorrhage volume removed over the next thirteen minutes.[5] This calculated hemorrhage volume was manually withdrawn from the iliac arterial access catheter over the course of this pre-hospital phase and stored for subsequent transfusion. If mean arterial pressure dropped below 30mmHg, hemorrhage was held until the blood pressure returned to 30mmHg with subsequent resumption of hemorrhage until the completion of the 20-minute pre-hospital phase. Then, during the Emergency Department phase of the study, 20% of total calculated blood volume was returned to the animal as fresh whole blood in keeping with the emerging concept of damage control resuscitation.

Continued hemorrhage

To ensure class IV hemorrhagic shock, all animals were subjected to ongoing hemorrhage at a rate of 0.15 mL/kg/min until proximal vascular control was achieved. As a result, total hemorrhage volumes varied slightly depending on the surgical adjunct used to simulate differences in time until proximal vascular control could be applied. For example, the thoracic

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aortic clamping group underwent 15 minutes of continued hemorrhage representing the time from arrival to the Emergency Department to time of descending thoracic aortic crossclamp placement. In contrast, the supra-celiac group underwent 25 minutes of continued hemorrhage simulating the additional time theoretically required for transport to the operating room. The direct vascular control group underwent 45 minutes of continued hemorrhage again simulating additional time for transport to the operating room and added time required to diagnose the source of hemorrhage and isolate the proximal aspect of the vessel. Finally, the endovascular aortic balloon occlusion group followed a similar time protocol to the thoracic aortic crossclamp group with 15 minutes of continued hemorrhage simulating an Emergency Department-based intervention.

Left anterolateral thoracotomy with thoracic aortic occlusion

Exposure of the thoracic aorta in animals randomized to thoracic aortic occlusion was achieved through a standard left anterolateral thoracotomy through the fourth intercostal space. The swine aorta was readily identified in all cases with its anterolateral relationship to the spine. Consequently, placement of an esophageal tube to facilitate identification of the aorta proved unnecessary. The aorta was occluded with an angled DeBakey aortic clamp and cessation of flow was confirmed distal to the clamp using Doppler ultrasound.

Laparotomy with supra-celiac aorta occlusion

Exposure of the supra-celiac aorta in animals randomized to trans-abdominal aortic control was established through an upper midline laparotomy incision. During model development, we found that the quickest access to the proximal abdominal aorta was achieved through left lateral to medial visceral rotation. As there are no lateral colonic attachments, this visceral rotation can be achieved with minimal dissection. The diaphragmatic fibers over the aorta were sharply divided

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to facilitate placement of the clamp. A straight DeBakey aortic clamp was advanced over the aorta until it contacted the spine posteriorly, and after application of the clamp, cessation of flow was confirmed by Doppler ultrasound.

Direct vascular control

Direct vascular control represented vascular control of the injury without previous clamping of the thoracic or abdominal aorta. Vascular control was simulated by cessation of ongoing hemorrhage from the iliac introducer sheath with subsequent placement of a temporary vascular shunt (TVS).

Endovascular aortic balloon occlusion

After exposure of the right iliac artery as described above, the right external iliac artery was cannulated with a short 8.5 Fr introducer sheath using a modified Seldinger technique then up-sheathed to a 16fr x 35cm introducer sheath. A 0.035 inch stiff Lunderquist wire was placed into the descending aorta and the 120 cm Coda® balloon catheter (Cook® Medical Inc., Bloomington, IN) was inserted using an over-the-wire technique. A hand injection aortogram was used for a roadmap, and the balloon was advanced to the just distal to the left subclavian artery takeoff from the thoracic aorta. The 32 mm balloon was inflated to an equilibrium pressure within the aorta to prevent rupture or dissection. Cessation of flow was confirmed with the left femoral arterial line. As described above, the balloon was inflated at 15 minutes post-hemorrhage time similar to the thoracic aortic occlusion timeline and was maintained in the inflated position for 30 minutes. A TVS was then placed at 45 minutes post-hemorrhage to simulate damage control of the injured iliac vessel.

Temporary vascular shunt insertion

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In all groups, damage control of the injured vessel was simulated and distal perfusion was restored with placement of a TVS (Sundt® 30 cm loop Carotid Endarterectomy Shunt, Integra LifeSciences, Plainsboro, NJ) into the right external iliac artery. The shunt was flushed with saline to avoid air embolism. The proximal control was temporarily released to forward flush the vessel. The distal control was let down to provide adequate back-bleeding in order to prevent shedding of thrombosis. The distal end of the vascular shunt was inserted first and blood was allowed to fill the shunt. The proximal end was then inserted and flow confirmed using Doppler ultrasound.

Surgical Intensive Care Unit management

All animals underwent continued resuscitation and monitoring after the above physiologic insult. Pre-defined resuscitation endpoints were used together with a resuscitation algorithm. Blood pressure was titrated to a goal mean arterial pressure of 60 mmHg. Initially, fluid boluses of warm 0.9% normal saline were used. If animals failed to respond to a fluid bolus or only responded transiently, norepinephrine (8mg/250cc preparation) was administered as an infusion to maintain a target mean arterial pressure.

Laboratory and histologic analysis

A complete laboratory panel was drawn at baseline and then after the pre-hospital hemorrhage phase at 10 minutes, 60 minutes, and every hour thereafter (Table 1). Post-mortem studies of tissue enzyme levels and histopathology were used to gauge end-organ damage in the heart and brain. Myocardial specimens were taken from the left ventricular free wall. Cardiac ischemia was assessed with tissue measurements of nitrotyrosine (ELISA and immunohistochemistry)[8] and myeloperoxidase (MPO) activity (ELISA and immunohistochemistry).[9]

For brain tissue sampling, a craniectomy was performed to confirm appropriate positioning of the brain tissue oxygenation probe in the right frontal lobe, and tissue samples were taken from the left brain to avoid errors associated with damage caused by the brain tissue oximetry probe. Sections were taken from the anterior, mid, and posterior cerebral cortex as well as the pons. Neurologic injury was measured by histologic analysis using a Fluoro-Jade B and terminal deoxynucleotidyl transferase-mediated dUTP end-labeling (TUNEL) staining.[6, 10]

Statistical analysis

Group means of single measures were compared by analysis of variance. Geometric means were compared for measures which appeared to be more normally distributed after log-transformation. The overall test of equality of means across groups was tested and considered significant if $p < 0.05$ before exploring differences between group pairs. For repeated measures, group comparisons were conducted using a mixed model with autoregressive first order covariance structure treating time as a categorical factor. SAS® 9.2 (SAS Institute Inc., Cary, NC) was used for all statistical calculations.

Results

In total, 30 animals (6 model development animals and 24 treatment animals) were utilized in this study (Fig. 3). Animal preparation, hemorrhage volume, open surgical technique and post-hemorrhage resuscitation protocols were developed using 4 initial model development animals. The endovascular approach was developed using 2 additional model development animals. Treatment animals subsequently underwent non-compressible hemorrhage with thoracotomy and thoracic aortic clamping (n=6), laparotomy and trans-abdominal supra-celiac clamping (n=6), direct vascular control without proximal aortic occlusion (n=6), and endovascular aortic balloon occlusion of the thoracic aorta (n=6). After model development, premature death occurred in 1 animal in the direct vascular control group during the ICU phase of the study.

Discussion

Non-compressible hemorrhage represents the leading cause of potentially preventable death on the modern battlefield.[11, 12] Combat casualty data from Operation Iraqi Freedom and Operation Enduring Freedom demonstrate a very high mortality rate associated with this pattern of injury. Despite aggressive utilization of pre-laparotomy thoracotomy which has now been pushed forward from the operating room to the Emergency Department in this clinical scenario, survival for patients in class IV hemorrhagic shock from penetrating trauma remain 5-15% at best.[1] This finding together with the relative logistical ease of rapidly moving patients to the operating room for definitive intervention and the emerging concept of endovascular aortic control directly motivated the development of this large animal to re-evaluate a host of intervention sequences and techniques in the setting of non-compressible hemorrhage.

The role of the resuscitative emergency thoracotomy with descending thoracic aortic occlusion for massive hemoperitoneum was first evaluated in a canine model in 1975[13] and was subsequently reported clinically by Ledgerwood and colleagues in 1976.[4] At the time, the traditional approach to the hypotensive patient with massive hemoperitoneum and subsequent tense abdominal distention was immediate laparotomy to identify and control the source of hemorrhage. 6 of 11 (55%) patients that underwent laparotomy were successfully resuscitated and injuries repaired and 4 of 11 (36%) survived to discharge. Ultimately, 11 of 29 (38%) patients that underwent pre-laparotomy thoracotomy survived the operative procedure and 7 of 29 (24%) survived to discharge. This clinical report ultimately transformed the practice of trauma surgery; however, with trauma operating rooms often located in close proximity to the trauma bay in many hospitals and with the revolution currently underway in resuscitation techniques, this approach bears re-evaluation.

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Others have described large animal models designed to evaluate related patterns of injury. Cho et al. established multiple-institutional large animal model incorporating hemorrhage, liver injury and femur fracture in a model which reliably results in the lethal triad of acidosis, coagulopathy and hypothermia.[14] This model demonstrated consistency of physiologic derangements across multiple institutions with a clinically appropriate mortality rate of 21%. However, such a model precludes a precise assessment of the effects of vascular control as the polytrauma design introduces multiple simultaneous variables which remain in flux through the resuscitation phase. While non-compressible hemorrhage is often associated with additional injuries, the ability to precisely control experimental variables in order to determine the optimal surgical control maneuver also has significant clinical value.

Kralovich et al. developed a swine (16-28 kg) model of pure hemorrhage with cardiovascular collapse treated with volume resuscitation, open cardiac massage, and endovascular aortic balloon occlusion.[15] These investigators found that aortic occlusion did not result in improved salvage and was associated with decreased left ventricular function, decreased oxygen consumption and systemic oxygen utilization, and increased systemic acidosis. Although similar to the model we report, several important differences bear highlighting. First, Kralovich used a venous hemorrhage source and did not incorporate any type of vascular repair or damage control intervention into their model. Hemorrhage was also precipitous in onset and then abruptly stopped in the pre-hospital phase which is not clinically realistic. Finally, the aortic occlusion arm of the study was by endovascular means only without confirmation of cessation of flow below the aortic balloon.

Although the model presented here permits precise evaluation of the clinical challenge of non-compressible torso hemorrhage, it is not without limitations. Because we sought to simulate the

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austere combat environment where whole blood is available but may be in limited quantity, crystalloid solution was incorporated into the resuscitation algorithm with numerous potentially deleterious side effects including upregulation of markers of cellular injury, immune activation, and exacerbation of trauma-related coagulopathy.[16] In addition, the overall survival was much greater than the anticipated survival demonstrated in the literature of 5-15% which may indicate that the physiologic challenge introduced by algorithm was overly cautious. One factor that could have influenced our overall survival rate is the fact that this model does not incorporate a splenectomy to avoid stress-induced autotransfusion as this practice has been recently called into question.[17] Lastly, due to limited previous development in this area, endovascular control was confined to devices designed to treat aneurysmal disease.

In summary, non-compressible hemorrhage represents a significant area of interest for the military community considering the high mortality of this injury pattern in combat casualties. This study reports a novel large animal model of class IV shock from non-compressible torso hemorrhage. The utility of this model lies in the ability to monitor the perfusion of vital organs during various vascular control methods while assessing the impact of these methods at both the global and microscopic levels within a clinically relevant timeframe of massive hemorrhage and subsequent resuscitation. Future studies utilizing this standardized model will permit the refinement of management strategies for trauma surgeons faced with this scenario and may motivate clinical trials to better define the role of trans-thoracic aortic control versus other means of staunching life-ending torso hemorrhage.

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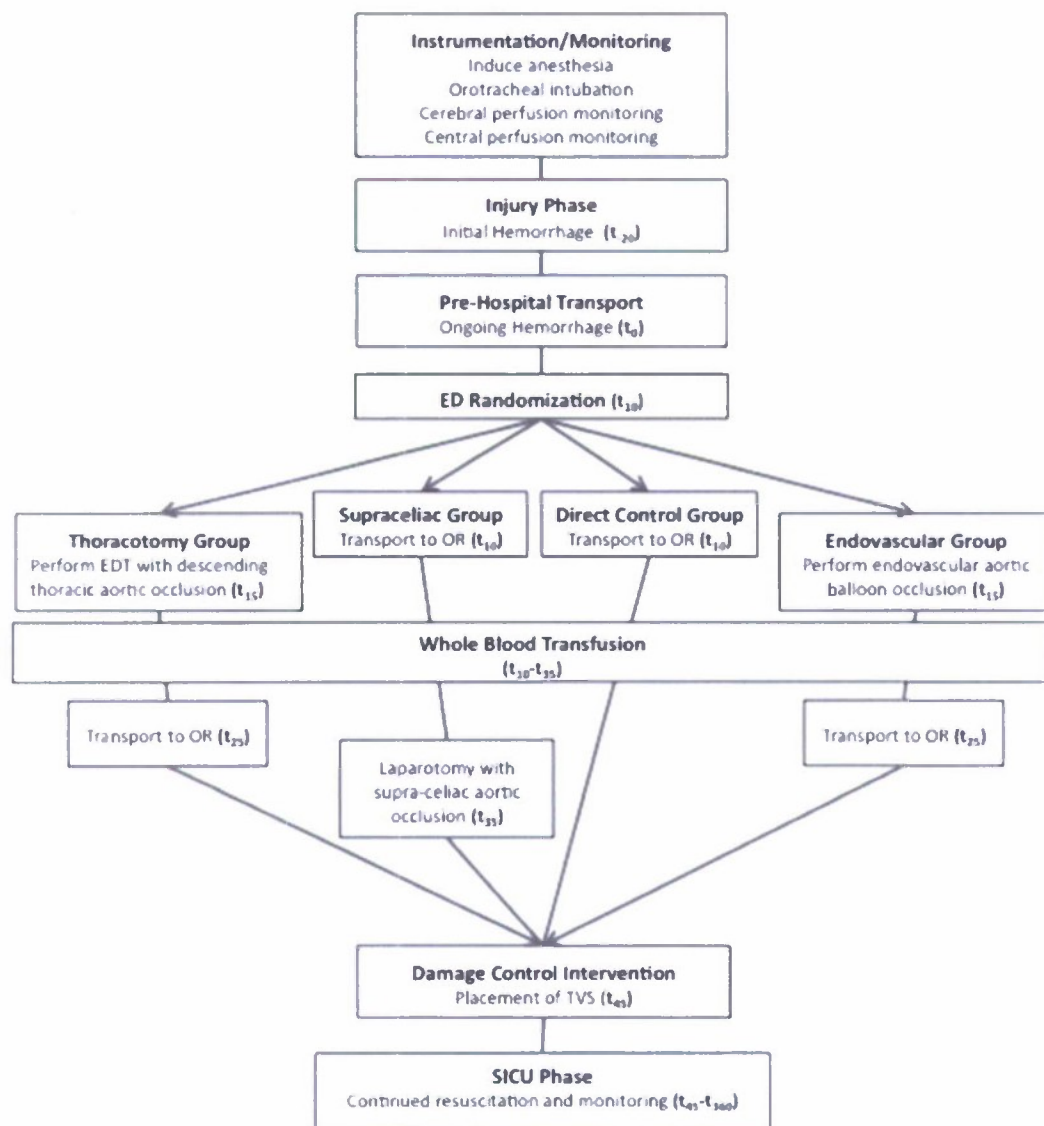
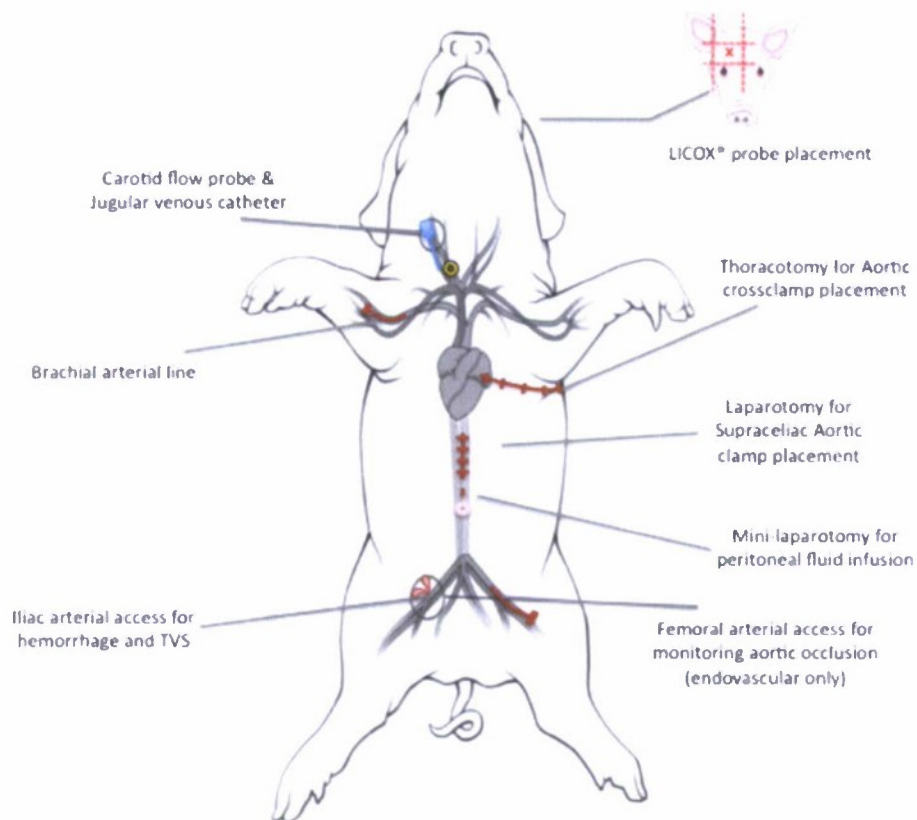Fig. 1. Protocol algorithm and timeline.

Fig. 2. Animal model with vascular access and monitors in place. Inset shows optimal placement of the intracranial LICOX® monitor for PbO$_2$ monitoring.
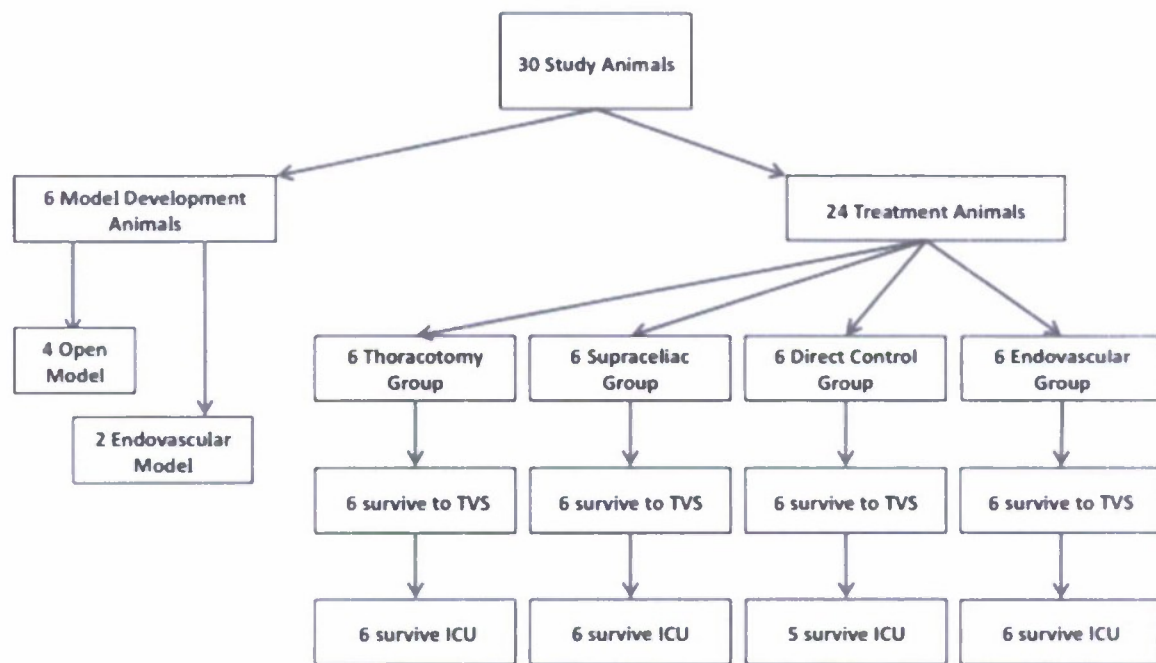
Fig. 3. Study animal distribution and survival by treatment group.

Table 1. Laboratory tests and tissue assays performed as part of this protocol.

Assay	Source		Collection Timing									
	S	T	Injury Phase (t_{-20})	ED (t_{10})	TVS (t_{45})	t_{60}	t_{120}	t_{180}	t_{240}	t_{300}	t_{360}	PM
ABG/VBG	x		x	x	x	x	x	x	x	x	x	
K⁺	x		x	x	x	x	x	x	x	x	x	
CPK	x		x	x	x	x	x	x	x	x	x	
CPK-MB	x		x	x	x	x	x	x	x	x	x	
Tn-T	x		x	x	x	x	x	x	x	x	x	
TEG	x		x	x	x	x	x	x	x	x	x	
LDH	x		x	x	x	x	x	x	x	x	x	
AST	x		x	x	x	x	x	x	x	x	x	
TNF-α	x		x		x						x	
IL-6	x		x		x						x	
Caspase-3	x		x		x						x	
Annexin V	x		x		x						x	
C MPO		x										x
C Nitro		x										x
B Jade		x										x
B TUNEL		x										x

S=serum sample; T=tissue sample; TVS=placement of temporary vascular shunt; PM=post mortem; TEG=thromboelastogram; C MPO=cardiac tissue myeloperoxidase activity; C Nitro=cardiac tissue nitrotyrosine levels; B Jade = brain tissue stained with Fluoro-Jade B; B TUNEL=brain tissue TUNEL staining.